June 2, 1953 H. L. DAVIS ET AL 2,640,752

PROCESS OF MAKING SUTURES

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Fig. 1.

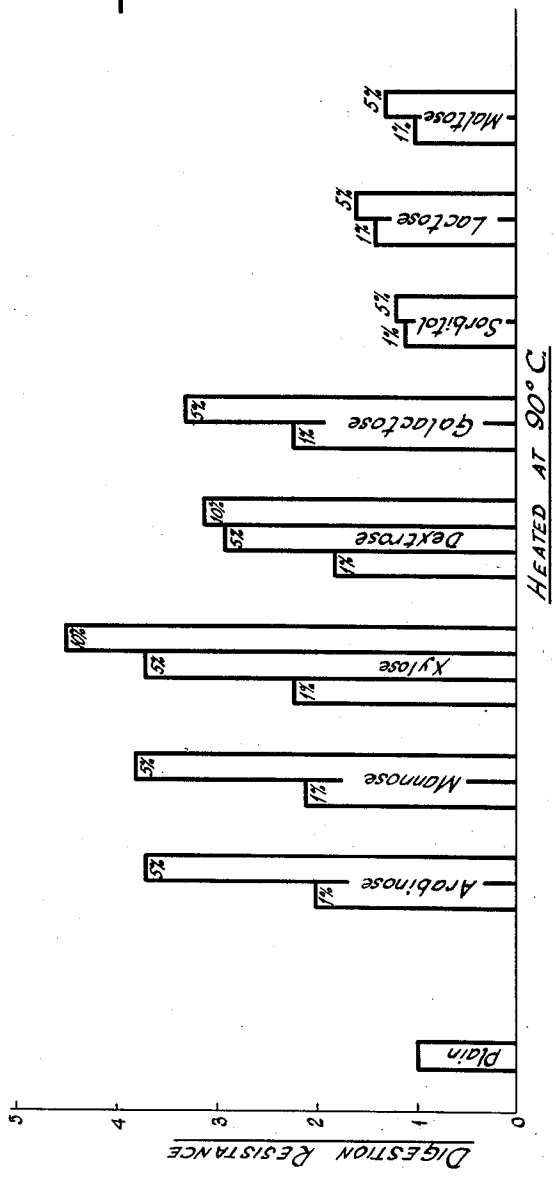

Fig. 2.

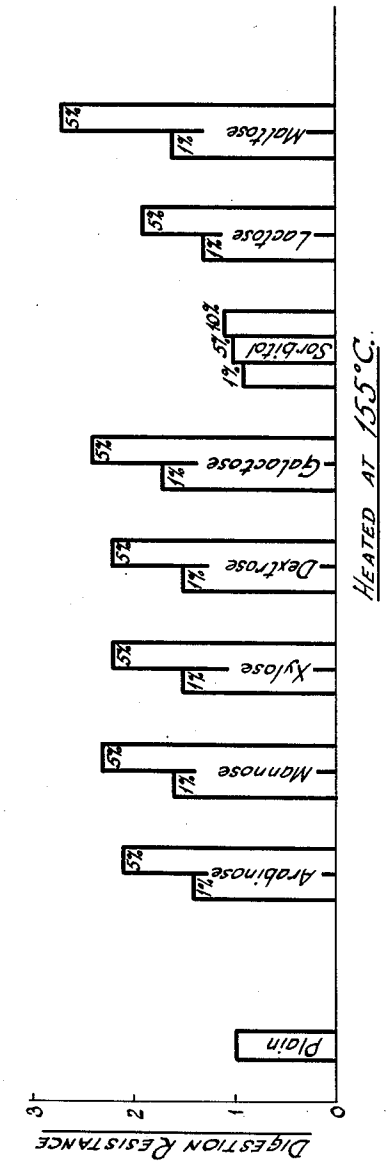

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Fig. 3.

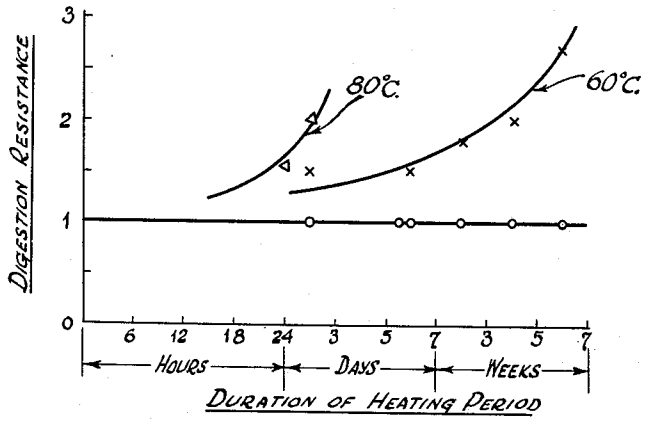
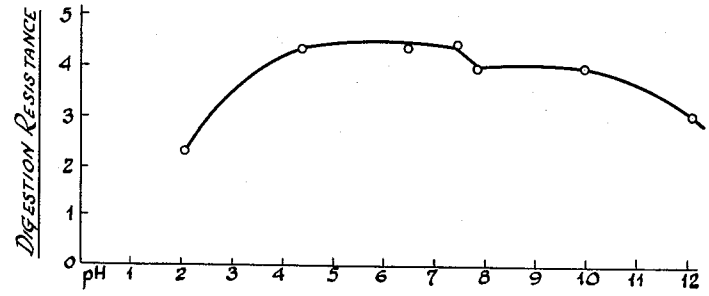


Fig. 4.



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Patented June 2, 1953

2,640,752

UNITED STATES PATENT OFFICE

2,640,752

PROCESS OF MAKING SUTURES

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Application November 30, 1949, Serial No. 130,296

9 Claims. (Cl. 8—94.11)

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This invention relates to an improved method for increasing the thermal and mechanical stability and digestion resistance in tissues and in the presence of hydrolytic enzymes, of the collagenous substances of animal tissues; and particularly relates to a method for increasing thermal and mechanical stability and the digestion resistance of surgical catgut sutures and ligatures.

Surgical catgut sutures and ligatures are prepared from the submucosa layer of sheep intestines which consists mainly of collagen. Animal hide used for leather manufacture is also mainly composed of collagen. In preparing sutures and ligatures, animal intestinal tubes are split longitudinally, cleaned and spun or twisted to form compact round strands. Such strands are called plain catgut and when implanted in certain animal tissues are normally digested and absorbed within a period of from 5 to 10 days. In something over 50% of the surgical procedures in which suturing and ligating is required, the surgeon demands that catgut suture and ligature strands retain useful tensile strength for increased periods in order to maintain cut tissue surfaces in apposition for a period exceeding that obtained from plain catgut. This is accomplished by increasing the ability of catgut to withstand the digestive forces in tissues as well as increased digestive forces under certain pathological conditions. This requires that the catgut have an increased resistance against the hydrolytic effect of the tissues which results from hydrolytic enzymes present in animal tissue.

The prior art has furnished a surgeon with catgut having a prolonged or increased holding power by subjecting catgut to chrome tanning, and by varying the degree of chrome tanning, digestion resistance and resistance to the hydrolytic attack of tissues are regulated. Chrome tanning was introduced by Lister over 60 years ago and great progress has been made in the procedure during recent years and although chrome-tanned catgut has received wide acceptance by members of the surgical profession, it is well recognized that certain disadvantages are inherent to chrome tanning and particularly to chrome-tanned catgut.

One disadvantage in the chrome tanning of collagenous materials and of catgut in particular is that the process must be carefully controlled with respect to a number of variables including concentration and acidity. Another disadvantage is that the chrome tanning liquor must be aged for a definite period of time before use since it has its maximum effectiveness and usefulness only during a limited range of aging time. Chrome tanning liquor requires the use of corrosive chemicals, such as sulfuric acid, sulfur dioxide and others which require precautions in handling and storage.

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One of the major disadvantages in chrome tanning catgut suture and ligature materials is that the molecules of the tanning agent in the tanning bath penetrate collagenous materials only with difficulty and this results in the deposition of a major amount of the tanning agent on the surface or to only a very slight extent beyond the surface of the collagenous material; such deposition blocks further infiltration of the chrome salt molecules into the collagenous substances. This phenomenon results in the surface strata of a collagenous suture or ligature being chrome tanned to a much greater extent than the core and consequently the core is less resistant to the hydrolytic action of tissues than is the surface. This property of chrome-tanned catgut strands can be readily observed for when such strands are exposed to proteolytic enzymes, digestion of the core of the strand proceeds much more rapidly and results in the formation of a hollow tube. If uniform tanning throughout the cross-section of catgut strands is desired, the thin flat ribbons of collagenous material, obtained by the longitudinal splitting of submucosa of sheep intestines, are tanned in that form and subsequently twisted into strands. This procedure is disadvantageous because extra handling is required and this results in substantial losses by weakening and breakage of the fragile flat ribbons.

Another disadvantage of chrome tanning is that this form of tanning is a reversible reaction, which may be demonstrated by immersing in a liquid both untanned and chrome-tanned collagenous material and by observing that after the lapse of a period of time, the chrome deposits originally present have migrated to some extent into the untanned material. It is known that when chrome-tanned catgut is implanted in animal tissues, molecules of the tanning agent

migrate into surrounding collagenous tissues, such as connective tissue, and act as a foreign body in the normally healthy connective tissue with the result that undesirable tissue irritation may appear.

In conventional procedures for preparing suture and ligature material, the strands must be sterilized by a separate heat treatment step after chrome tanning. Before heat sterilization the strands are dehydrated by the application of heat at about 100° C. The sterilization step is called cumolization and consists in heating chrome-tanned strands immersed in a liquid aliphatic hydrocarbon such as naphtha in glass tubes. After cooling, the hydrocarbon is drained from the tube and tubing fluid is added; the tube is finally sealed.

It has now been found that collagen, and surgical catgut strands in particular, may be given an increased resistance to digestion by the hydrolytic enzymes present in normal tissue by a treatment with reducing sugars. It has been discovered that the treatment of collagen with such reducing sugars also acts to raise its thermal and mechanical stability.

An object of this invention is to provide an improved process for increasing the resistance of collagenous materials to the hydrolytic enzymes normally present in animal tissues.

Another object of this invention is to provide an improved process for increasing the resistance of collagenous materials to the hydrolytic enzymes present in animal tissues and to raise the thermal and mechanical stability of said collagenous materials.

Another and further object of this invention is to provide a method for increasing the resistance of collagenous suture and ligature materials to the hydrolytic enzymes of normal animal tissue by reacting said collagenous materials with a reducing sugar or sugars.

Still another object of this invention is to provide a method for increasing the thermal and mechanical stability of collagenous strands as well as their resistance to hydrolytic enzymes present in normal tissue by treating such strands with reducing sugars at a pH within the range of from 4 to 10, and then subjecting the impregnated material to a heat treatment at an elevated temperature.

Another object of this invention is to provide improved collagenous suture and ligature material having increased thermal and mechanical stability and increased resistance to hydrolytic enzymes normally present in animal tissue.

The objects of this invention are accomplished by immersing collagenous materials, and collagenous ribbons and spun strands in particular, in a solution of a reducing sugar or sugars, removing the collagenous materials from the solution, drying the materials, and subjecting them to a heat treatment.

Because of the small size of the molecules of the reducing sugars, they readily move through the outer strata and freely and completely penetrate and impregnate the collagenous material, and strands of suture and ligature material in particular, when the material is immersed in a solution of one or more reducing sugars. The preferred solvent for the reducing sugar or sugars is water but mixtures of lower aliphatic alcohols and water are satisfactory; including 95% ethanol. Immersion in a solution of reducing sugar or sugars results in a uniform distribution of the reducing substance throughout the collagenous material. The strands are removed from the solution, air-dried, and then heated to an elevated temperature for a period of time ranging from an hour to more than a day. Impregnated strands must be dried before they are heated at elevated temperatures in order not to weaken the strands; air drying at a temperature between 20° C. and 40° C. is preferred. Heating of the impregnated and dried collagenous material results in a reaction between the reducing sugar and the collagenous substance, which reaction is superficially manifested by a brown coloration. The intensity of the coloration is an indication of the extent to which the reaction between the reducing sugar and the collagenous substance has progressed. The reaction between collagenous material and reducing sugars will take place even at room temperatures but only very slowly and therefore it is preferred that the reaction be accelerated by heat. It is preferred that dried impregnated collagenous strands be heated at a temperature within the range of from 70° C. to 156° C. to effect the reaction between the sugar and the collagenous substance. The desired reaction between a reducing sugar and collagenous material does not take place when the collagenous material is heated while immersed in an aqueous solution of a reducing sugar.

The heating period to which dried collagenous material impregnated with reducing sugars is subjected may be adjusted and correlated with the concentration of the solution of reducing sugar in order that the finished material has the ability to withstand the hydrolytic action of animal tissue for a desired period of time and maintain apposition of cut tissue surfaces. Sterilization of collagenous material may coincide with the heating period by adjustment of the concentration of the solution of reducing sugar with the length of time and the temperature to which impregnated material is subjected during the heating period. The impregnated collagenous material may be cumolized and during this process the reaction between the reducing sugar and the collagenous material is also accomplished.

The pH of the solution of the reducing sugar may be adjusted by buffering over a range of from 4 to 10, but it is preferred that the pH be from 4 to 7.

Reducing sugars which may be used in the reaction with collagenous materials include all sugars which are classified as reducing sugars. Sugars which have been found to satisfactorily react with collagenous materials include the monosaccharides, xylose, arabinose, fructose, glucose, galactose, ascorbic acid, and mannose; and the disaccharides, maltose and lactose.

The effect on increased resistance to hydrolytic or proteolytic enzymes of the treatment of collagenous material with reducing sugars is measured by determining the rate of digestibility at body temperature of such material in aqueous solutions of enzymes such as pepsin, trypsin, and papain; and by the rate of hydrolysis in acid buffer systems of a low pH at 100° C. In determining digestion resistance of impregnated and unimpregnated strands of suture and ligature material which have been subjected to a heat treatment after immersion in a solution of a reducing sugar or sugars, seven-inch lengths of the strands are tied to form loops and the loops are hung in an enzyme solution under the constant tension obtained by attaching a twenty-gram weight to one end of the loop, the other end of the loop being maintained in a stationary position.

In conducting the tests, automatic recording devices record the time required to digest or hydrolyze the strands to such an extent that the twenty-gram weight will cause the looped strand to break, i. e., go to twenty-gram strength. By comparing the results of such tests with results obtained by using unimpregnated collagenous strands which have been subjected to the same heating period as the treated strands, an estimate of the increased resistance to hydrolytic enzymes present in animal tissues may be made.

The following are examples of enzyme solutions used for determining digestion resistance and methods for their preparation. A buffered solution of pepsin is prepared by dissolving one gram of the spongy granular enzyme in 100 cc. of a buffer solution, prepared by dissolving 75 grams of potassium chloride and 97 cc. of concentrated hydrochloric acid in distilled water and bringing the volume up to 20 liters with distilled water. The pH of this buffer solution is 1.35 and the pH of the buffered enzyme solution is 1.5.

A buffered solution of papain is prepared by dissolving three grams of the enzyme in 100 cc. of a buffer solution containing 7.6 grams of thiourea. Four cc. of 5% sodium cyanide are added to 96 cc. of the above solution of papain in the buffer solution just prior to use; the final pH of this solution is 7.2.

The following examples are given to illustrate specific embodiments of the invention but it is not intended that they should limit or delineate the scope of the invention in any way.

Example I

Collagenous suture strands weighing 22.5 grams were immersed for 20 hours at 32° F. and under occasional agitation in a solution containing 20 grams of dextrose dissolved in one liter of water. The solution had a pH of 7.3. The strands were removed from the solution, rinsed with water, and dried. The dried strands were heated for 1.5 hours at a temperature of 156° C. Unimpregnated suture strands required a weight of 12 pounds and impregnated suture strands required a weight of 13.0 pounds to effect breakage. Unimpregnated suture strands required 4.5 hours and impregnated suture strands required 10 hours to go to twenty-gram strength in a solution of papain. Both impregnated and unimpregnated strands had an average diameter of 19.5 thousandths of an inch.

Example II

Collagenous suture strands weighing five grams were immersed for 22 hours at room temperature and with occasional agitation in a solution containing 17.5 grams of dextrose and 3 grams of alum dissolved in one liter of water. The pH of the treating solution was 4.2. The strands were removed from the solution, rinsed with water, dried and heated at a temperature of 156° C. for a period of one hour. Unimpregnated suture strands required a weight of 12.9 pounds and impregnated strands required a weight of 14.0 pounds to effect breakage. Unimpregnated suture strands went to twenty-gram strength in 4.5 hours and impregnated suture strands in 9.5 hours immersion in a solution of papain. Both impregnated and unimpregnated strands had an average diameter of 20.1 thousandths of an inch.

Example III

Collagenous suture strands weighing 45 grams were immersed in a solution of 90 grams of dextrose, 27 grams of alum, and 18 cc. of formalin dissolved in 9 liters of water. The suture strands were allowed to remain in the solution for 20 hours during which time the solution was at room temperature and was occasionally agitated. The strands were removed, rinsed with water, dried, and heated at a temperature of 155° C. for a period of one hour. Before impregnation the suture strands required a weight of 12 pounds and after impregnation a weight of 14.3 pounds to effect breakage. Both impregnated and unimpregnated strands had an average diameter of 18.3 thousandths of an inch. The pH of the sugar solution was 4.2. Unimpregnated suture strands went to 20-gram strength in a solution of papain in 4.5 hours; impregnated strands required 15.4 hours to go to 20-gram strength.

Example IV

Collagenous suture strands weighing 20 grams were immersed for 20 hours at room temperature in 2.5 liters of an aqueous solution containing 125 grams of lactose. During the time of immersion the solution was occasionally agitated. The pH of the solution was 7.1. The strands were removed, rinsed with water, dried, and gradually brought to a temperature of 90° and held at that temperature for 5 hours and subsequently heated at a temperature of 156° C. for one hour. Unimpregnated strands required a weight of 12.0 pounds and impregnated strands a weight of 12.6 pounds to effect breakage. Unimpregnated strands required 5 hours and impregnated strands required 10.1 hours immersion in a solution of papain to go to twenty-gram strength. Both impregnated and unimpregnated strands had an average diameter of 18.3 thousandths of an inch.

Example V

Collagenous suture strands weighing 20 grams were immersed in 2 liters of an aqueous solution of 20 grams of xylose for 24 hours at room temperature. The pH of the solution was 7.2. The solution was occasionally agitated during the time of immersion and at the end of this time the strands were removed from the solution, air dried, gradually brought to a temperature of 90° over a period of 10 hours and finally heated to 156° C. for one hour. Both impregnated and unimpregnated strands had an average diameter of 18.5 thousandths of an inch. Unimpregnated strands required a weight of 12.3 pounds and impregnated strands a weight of 12.5 pounds to effect breakage. Unimpregnated strands required 5 hours and impregnated strands 9 hours immersion in a solution of papain to go to twenty-gram strength.

Example VI

A series of suture strands were immersed in solutions of mannose and arabinose of varying concentrations, the sugar solutions having a pH of 6.8. The time of immersion was 24 hours at room temperature with occasional agitation. The strands were removed from the solutions and air dried. All of the strands were brought to a temperature of 90° C. during a period of ten hours and part of the strands were tested for digestion resistance in a solution of papain. A portion of the strands were heated for an additional time of one hour at 155° C. and then tested for digestion resistance in a solution of papain. The results are given in the table below in hours required for impregnated and unimpregnated strands to go to twenty-gram strength in a solution of papain. Both impregnated and un-

impregnated strands had an average diameter of 18.5 thousandths of an inch.

	Temperature	
	90° C.	155° C.
Unimpregnated Control	4.0	2.5
Impregnated with Mannose, 1%	5.5	4.4
Impregnated with Mannose, 5%	8.0	7.9
Impregnated with Arabinose, 1%	4.3	3.9
Impregnated with Arabinose, 4%	6.1	5.3

The results of this series of experiments show the effect of varying the concentration of the sugar solution and the temperature on digestion resistance. Digestion resistance is increased by increasing the concentration of sugar in the immersing solution but an increase in temperature alone does not increase the apparent digestion resistance in papain solution in these examples. The effect of heat alone on collagenous materials reduces digestion resistance against a papain solution, and this is particularly marked at higher temperatures.

Collagenous strands, treated according to the procedure in Example VI, showed an increase in digestion resistance in pepsin solution with an increase in temperature during the heating period. Unimpregnated collagenous strands also showed an increase in digestion resistance in pepsin solution with an increase in temperature during the heating period.

Although all the examples show the treatment of collagenous material in the form of strands, the process is equally effective when applied to ribbons of such material. Moist strands and fully hydrated ribbons of collagenous material, as obtained from intestines, may be immersed directly in the solution of reducing sugar or sugars or they may be dried and stored before immersion. Impregnated collagenous material may be stored for indefinite periods of time without deleterious effects thereon before proceeding with the heat treatment to effect the reaction between the collagenous material and the reducing sugar or sugars.

Fig. 1 shows by graphical representation the digestion resistance of collagenous strands having an average diameter within the range of from 18 to 22 thousandths of an inch before immersion in the enzyme solution. In all instances the strands were immersed in sugar solution at about pH 6.8 for twenty-four hours at room temperature with occasional agitation. After the immersion period the strands were air dried and heated at 90° C. for five hours. In all instances digestion resistance was measured as the time required for a loop of a seven-inch length of strand to break under a load of 20 grams when immersed in a 3% aqueous solution of papain buffered at pH 7.2 and maintained at body temperature. In Fig. 1 the time of breakage for an impregnated strand subjected to the above treatment as regards all steps except immersion in the sugar solution is taken as a unit of digestion resistance.

Fig. 2 shows by graphical representation the digestion resistance of collagenous strands, having an average diameter within the range of from 18 to 22 thousandths of an inch before immersion in the enzyme solution. These strands were treated according to the same procedure as the strands for which the digestion resistance is given in Fig. 1, except as to the heat treatment, which in this case was made by bringing the air-dried strands to 90° C. during a period of twelve hours followed by a heating period of one hour at 155° C. In Fig. 2 the time of breakage for an unimpregnated strand subjected to the above treatment as regards all steps except immersion in the sugar solution is taken as a unit of digestion resistance.

The effect of the process of this invention of increasing digestion resistance to proteolytic enzymes is stronger when collagenous materials are impregnated with pentoses than when they are impregnated with hexoses. Monosaccharides produce a greater digestion resistance than disaccharides when lower curing temperatures are used. This is illustrated in Fig. 1, in which the heating temperature was 90° C. As the heating temperature is raised, all reducing sugars have a similar effect on digestion resistance of collagenous strands to proteolytic enzyme solutions. This is illustrated in Fig. 2.

Resistance to digestion by proteolytic enzymes in a buffered solution is increased with increasing concentration of reducing sugars in the treating bath and the reaction between the reducing sugar and the collagenous material is accelerated as the heating temperature is increased.

Fig. 3 shows by graphical representation the digestion resistance of a series of collagenous strands immersed in a 5% aqueous dextrose solution, air-dried, and heated at 37° C., 60° C., and 80° C., respectively, for periods of time ranging from a few hours to several weeks. In all instances digestion resistance is measured as the time required for a loop of a seven-inch length of strand to break under a load of twenty grams when immersed in a 3% aqueous solution of papain buffered at pH 7.2 and maintained at body temperature.

The results presented in Fig. 3 show that the rate of reaction between collagenous strands and reducing sugars is very slow at 60° C. and below. At this temperature several days or even weeks may be required to appreciably increase the resistance to hydrolytic or proteolytic enzymes in a buffered solution. Heating impregnated strands for a period as long as six weeks at 26° C. or 37° C. does not increase the digestion resistance of such strands above that of unimpregnated strands heated at 26° C. or 37° C. for six weeks. The horizontal line drawn through unit one of digestion resistance in Fig. 3 represents the digestion resistance for both impregnated and unimpregnated strands heated at 26° C. and 37° C. for six weeks. As the temperature is raised above 60° C., the time of reaction becomes a matter of a few hours. In Fig. 3 the time of breakage for an unimpregnated strand subjected to the above treatment as regards all steps except immersion in the sugar solution is taken as a unit of digestion resistance. The unit of digestion resistance is substantially the same for unimpregnated strands heated at 26° C., 37° C., 60° C., and 80° C., respectively. At any given heating temperature the degree of digestion resistance is increased by increasing the heating time. The heating of impregnated or unimpregnated collagenous strands above 160° C. even for short periods of time results in marked loss of strength. Heating periods of from five days to 1.5 weeks at 70° C., from five to twelve hours at 90° C., from one to 1.5 hours at 156° C., and from five to twelve hours at 90° C. followed by one hour at 156° C. have been found to yield suture and ligature strands with good digestion resistance, and thermal and mechanical stability.

Fig. 4 shows by graphical representation the results of a series of experiments in which collagenous strands were immersed in a 5% aqueous dextrose solution at varying levels of pH for a period of four hours followed by air drying the strands and heating them at a temperature of 155° C. for a period of 1.5 hours. In Fig. 4 the pH of the solution of reducing sugar is plotted against digestion resistance, measured as the time required for a looped seven-inch length of impregnated and heated collagenous strand to break under a load of twenty grams when immersed in a 3% aqueous solution of papain buffered at pH 7.2 and maintained at body temperature. A unit of digestion resistance in Fig. 4 is the time of breakage for a strand subjected to the above treatment as regards all steps except immersion in the sugar solution.

The results shown in Fig. 4 show that the rate of digestion of collagenous strands impregnated with dextrose is comparatively independent of the pH of the sugar solution over a range of pH of from 4 to 10. At a pH of the impregnating bath of less than 4 or greater than 10 the degree of digestion resistance is markedly less, which may be due to the deleterious effect of strongly acid or alkaline conditions on collagenous substances.

While the invention has been described in detail according to the preferred manner of carrying out the process and yielding the products, it will be obvious to those skilled in the art, after understanding the invention, that changes and modifications may be made therein without departing from the spirit or scope of the invention, and it is intended in the appended claims to cover all such changes and modifications.

What is claimed is:

1. A process for increasing the thermal stability, mechanical stability, and resistance to digestion by proteolytic enzymes of filamentous collagen sutures which comprises the steps of; impregnating filamentous collagen with at least one reducing sugar by immersing said filamentous collagen in a reducing sugar solution, removing and drying the impregnated filamentous collagen at a temperature not greater than 40° C., and heating the dry impregnated filamentous collagen in the absence of water at a temperature within the range of from 70° C. to 156° C.

2. A process according to claim 1 in which the sugar is glucose.

3. A process according to claim 1 in which the sugar is maltose.

4. A process according to claim 1 in which the sugar is lactose.

5. A process for increasing the thermal stability, mechanical stability, and resistance to digestion by proteolytic enzymes of filamentous collagen sutures which comprises the steps of; impregnating filamentous collagen with at least one reducing sugar by immersing said filamentous collagen in an aqueous reducing sugar solution having a pH within the range of from 4 to 10, removing and drying the impregnated filamentous collagen at a temperature not greater than 40° C., and heating the dry impregnated filamentous collagen in the absence of water at a temperature within the range of from 70° C. to 156° C.

6. A process according to claim 5 in which the sugar is glucose.

7. A process according to claim 5 in which the sugar is maltose.

8. A process according to claim 5 in which the sugar is lactose.

9. A process for increasing the thermal stability, mechanical stability, and resistance to digestion by proteolytic enzymes of filamentous collagen sutures in the form of strands which comprises the steps of; impregnating filamentous collagen in the form of strands with at least one reducing sugar by immersing said filamentous collagen in an aqueous reducing sugar solution having a pH within the range of from 4 to 10, removing and drying the impregnated filamentous collagen at a temperature within the range of from 20° C. to 40° C., and heating the dry impregnated filamentous collagen in the absence of water and at a temperature within the range of from 70° C. to 156° C.

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